AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows:

(1) Please delete paragraph [0066] of the originally filed specification and replace it with the following paragraph:

[0066] In a particularly preferred embodiment, the positively charged backbone is a polypeptide having branching groups (also referred to as efficiency groups) comprising $-(gly)_{n1}-(arg)_{n2}$ SEQ ID NO. 1, HIV-TAT or fragments thereof, or the protein transduction domain of Antennapedia, or a fragment thereof, in which the subscript n1 is an integer of from 0 to 20, more preferably 0 to 8, still more preferably 2 to 5, and the subscript n2 is independently an odd integer of from about 5 to about 25, more preferably about 7 to about 17, most preferably about 7 to about 13. Still further preferred are those embodiments in which the HIV-TAT fragment has the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$ SEQ ID NO. 2, $(gly)_p$-YGRKKRRQRRR-$(gly)_q$ SEQ ID NO. 3 or $(gly)_p$-RKKRRQRRR-$(gly)_q$ SEQ ID NO. 4 wherein the subscripts p and q are each independently an integer of from 0 to 20 and the fragment is attached to the backbone via either the C-terminus or the N-terminus of the fragment. Preferred HIV-TAT fragments are those in which the subscripts p and q are each independently integers of from 0 to 8, more preferably 2 to 5. In another preferred embodiment the positively charged side chain or branching group is the Antennapedia (Antp) protein transduction domain (PTD), or a fragment thereof that retains activity. Preferably the positively charged carrier includes side-chain positively charged branching groups in an amount of at least about 0.05%, as a percentage of the total carrier weight, preferably from about 0.05 to about 45 weight %, and most preferably from about 0.1 to about 30 weight %. For positively charged branching groups having the formula--$(gly)_{n1}$-$(arg)_{n2}$ SEQ ID NO. 1, the most preferred amount is from about 0.1 to about 25%.

(2) Please delete paragraph [0067] of the originally filed specification and replace it with the following paragraph:

[0067] In another particularly preferred embodiment, the backbone portion is a polylysine and positively charged branching groups are attached to the lysine sidechain amino groups. The polylysine used in this particularly preferred embodiment has a molecular weight of from about 10,000 to about 1,500,000, preferably from about 25,000 to about 1,200,000, and most preferably from about 100,000 to about 1,000,000. It can be any of the commercially available (Sigma Chemical Company, St. Louis, Mo., USA) polylysines such as, for example, polylysine having MW>70,000, polylysine having MW of 70,000 to 150,000, polylysine having MW 150,000 to 300,000 and polylysine having MW>300,000. The selection of an appropriate polylysine will depend on the remaining components of the composition and will be sufficient to provide an overall net positive charge to the composition and provide a length that is preferably from one to four times the combined length of the negatively charged components. Preferred positively charged branching groups or efficiency groups include, for example, -gly-gly-gly-arg-

arg-arg-arg-arg-arg-arg (-Gly_3Arg_7 SEQ ID NO. 5) or HIV-TAT. In another preferred embodiment the positively charged backbone is a long chain polyalkyleneimine such as a polyethyleneimine, for example, one having a molecular weight of about 1,000,000.

(3) Please delete paragraph [0069] of the originally filed specification and replace it with the following paragraph:

[0069] In one embodiment of the invention, only a positively charged carrier that has positively charged branching groups is necessary for transdermal delivery of the active substance (e.g. a biologically active agent, or imaging/targeting agent). In one embodiment of this case the positively charged carrier is a polypeptide (e.g., lysine, arginine, ornithine, homoarginine, and the like) having multiple positively charged side-chain groups, as described above. Preferably, the polypeptide has a molecular weight of at least about 10,000. In another embodiment, the positively charged carrier is a nonpeptidyl polymer such as a polyalkyleneimine having multiple positively charged side-chain groups having a molecular weight of at least about 100,000. Such polyalkyleneimines include polyethylene- and polypropyleneimines. In either instance, for use as the sole necessary agent for transdermal delivery the positively charged carrier molecule includes positively charged branching or efficiency groups, comprising $(gly)_{n1}$-$(arg)_{n2}$, SEQ ID NO. 1, in which the subscript n1 is an integer of from 0 to 20 more preferably 0 to 8, still more preferably 2 to 5, and the subscript n2 is independently an odd integer of from about 5 to about 25, more preferably from about 7 to about 17, and most preferably from about 7 to about 13, HIV-TAT or fragments thereof, or Antennapedia PTD or a fragment thereof. Preferably the side-chain or branching groups have the general formula -$(gly)_{n1}$-$(arg)_{n2}$ SEQ ID NO. 1 as described above. Other preferred embodiments are those in which the branching or efficiency groups are HIV-TAT fragments that have the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$ SEQ ID NO. 2, $(gly)_p$-YGRKKRRQRRR-$(gly)_q$ SEQ ID NO. 3, or $(gly)_p$-RKKRRQRRR-$(gly)_q$ SEQ ID NO. 4, wherein the subscripts p and q are each independently an integer of from 0 to 20 and the fragment is attached to the carrier molecule via either the C-terminus or the N-terminus of the fragment. The side branching groups can have either the D- or L-form (R or S configuration) at the center of attachment. Preferred HIV-TAT fragments are those in which the subscripts p and q are each independently integers of from 0 to 8, more preferably 2 to 5. Other preferred embodiments are those in which the branching groups are Antennapedia PTD groups or fragments thereof that retain the group's activity. These are known in the art, for instance, from Console et al., J. Biol. Chem. 278:35109 (2003).

(4) Please delete paragraph [0070] of the originally filed specification and replace it with the following paragraph:

[0070] In a particularly preferred embodiment, the carrier is a polylysine with positively charged branching groups attached to the lysine side-chain amino groups. The polylysine used in this particularly preferred embodiment can be any of the commercially available (Sigma Chemical Company, St. Louis, Mo., USA, e.g.) polylysines such as, for example, polylysine having MW>70,000, polylysine having MW of 70,000 to 150,000, polylysine having MW

150,000 to 300,000 and polylysine having MW>300,000. However, preferably the polylysine has MW of at least about 10,000. Preferred positively charged branching groups or efficiency groups include, for example, -gly-gly-gly-arg-arg-arg-arg-arg-arg-arg (-Gly_3Arg_7 SEQ ID NO. 5), HIV-TAT or fragments of it, and Antennapedia PTD or fragments thereof.

(5) Please delete paragraph [0074] of the originally filed specification and replace it with the following paragraph:

[0074] The imaging moieties and targeting moieties can themselves be small anions in the absence of a negatively charged polymer. The imaging moieties, targeting moieties and therapeutic agents can also be themselves covalently modified to afford sufficient surface negatively charged moieties for ionic complexation with the positively-charged backbones as will be readily apparent to one skilled in the art. In both of these cases, the substance or a derivative thereof has sufficient negative charge to associate with the positively charged carriers of the present invention non-covalently. ~~The term "sufficient" in this context refers to an association that can be determined for example by change in particle sizing or functional spectrophotometry versus the components a~~

(6) Please delete paragraph [0093] of the originally filed specification and replace it with the following paragraph:

[0093] In yet another aspect, the present invention provides compositions comprising a non-covalent complex of a positively-charged backbone having at least one attached efficiency group and In this aspect of the invention, the positively-charged backbone can be essentially any of the positively-charged backbones described above, and will also comprise (as with selected backbones above) at least one attached efficiency group. Suitable efficiency groups include, for example, $(Gly)_{n1}$-$(Arg)_{n2}$ SEQ ID NO. 6 wherein the subscript n1 is an integer of from 3 to about 5, and the subscript n2 is independently an odd integer of from about 7 to about 17; or TAT domains. For example, the TAT domains may have the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$ SEQ ID NO. 2, $(gly)_p$-YGRKKRRQRRR-$(gly)_q$ SEQ ID NO. 3 or $(gly)_p$-RKKRRQRRR-$(gly)_q$ SEQ ID NO. 4 wherein the subscripts p and q are each independently an integer of from 0 to 20 and the fragment is attached to the carrier molecule via either the C-terminus or the N-terminus of the fragment. The side branching groups can have either the D- or L-form (R or S configuration) at the center of attachment. Preferred HIV-TAT fragments are those in which the subscripts p and q are each independently integers of from 0 to 8, more preferably 2 to 5.

(7) Please delete paragraph [0121] of the originally filed specification and replace it with the following paragraph:

[0121] The positively charged backbone was assembled by covalently attaching - Gly_3Arg_7 (SEQ ID NO. 5) to polylysine MW 150,000 via the carboxyl of the terminal glycine to free amines of the lysine sidechains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is covalently attached to a - Gly_3Arg_7 (SEQ ID NO. 5). The modified backbone was designated "KNR2" to denote a second size of the peptidyl carrier. The control polycation was unmodified polylysine (designated "K2", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot. An additional control polycation, Superfect.RTM. (Qiagen) which is an activated dendrimer-based agent, was selected as a reference for high in vitro transfection rates (i.e. simultaneous positive control and reference for state-of-the art efficiency versus toxicity in vitro).

(8) Please delete paragraph [0132] of the originally filed specification and replace it with the following paragraph:

[0132] The positively charged backbone was assembled by covalently attaching - Gly_3Arg_7 (SEQ ID NO. 5)to polylysine (MW 150,000) via the carboxyl of the terminal glycine to free amines of the lysine sidechains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is covalently attached to a - Gly_3Arg_7 (SEQ ID NO. 5). The modified backbone was designated "KNR2" as before. The control polycation was unmodified polylysine (designated "K2", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot. An additional control polycation, Superfect (Qiagen) which is an activated dendrimer-based agent, was selected as a reference for high transfection rates (i.e. simultaneous positive control and reference for state-of-the art efficiency versus toxicity in vitro).

(9) Please delete paragraph [0142] of the originally filed specification and replace it with the following paragraph:

[0142] The positively charged peptidyl backbone was assembled by covalently attaching -Gly_3Arg_7 (SEQ ID NO. 5) to polylysine (MW 150,000) via the carboxyl of the terminal glycine to free amines of the lysine sidechains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is covalently attached to a -Gly_3Arg_7 (SEQ ID NO. 5)). The modified backbone was designated "KNR2". The control polycation was unmodified polylysine (designated "K2", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot.

(10) Please delete paragraph [0149] of the originally filed specification and replace it with the following paragraph:

[0149] The positively charged backbone was assembled by covalently attaching-Gly_3Arg_7 (SEQ ID NO. 5) to polyethyleneimine (PEI, MW 1,000,000) via the carboxyl of the terminal glycine to free amines of the PEI sidechains at a degree of saturation of 30% (i.e., 30 out of each 100 lysine residues is covalently attached to a -Gly_3Arg_7 (SEQ ID NO. 5)). The modified backbone was designated "PEIR" to denote the large nonpeptidyl carrier. The control polycation was unmodified PEI (designated "PEI", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot.

(11) Please delete paragraph [0157] of the originally filed specification and replace it with the following paragraph:

[0157] The positively charged backbone was assembled by covalently attaching -Gly_3Arg_7 (SEQ ID NO. 5) to polylysine (MW 112,000) via the carboxyl of the terminal glycine to free amines of the lysine side chains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is covalently attached to a -Gly_3Arg_7 (SEQ ID NO. 5)). The modified backbone was designated "KNR". The control polycation was unmodified polylysine (designated "K", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot.

(12) Please delete the first eight lines of paragraph [0166] of the originally filed specification and replace it with the following text:

[0166] The positively charged backbone was again assembled by covalently attaching -Gly_3Arg_7 (SEQ ID NO. 5) to polylysine MW 112,000 via the carboxyl of the terminal glycine to free amines of the lysine side chains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is covalently attached to a -Gly_3Arg_7 (SEQ ID NO. 5)). The modified backbone was designated "KNR". Control polycation was unmodified polylysine (designated "K", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot. The same botulinum toxin therapeutic agent was used as in Example 5, and was prepared in the same manner. Samples were prepared as follows:

(13) Please delete paragraph [0172] of the originally filed specification and replace it with the following paragraph:

[0172] The positively charged backbone was assembled by covalently attaching -Gly_3Arg_7 (SEQ ID NO. 5) to polyethyleneimine (PEI) MW 1,000,000 via the carboxyl of the terminal glycine to free amines of the PEI side chains at a degree of saturation of 30% (i.e., 30 out of each 100 lysine residues is covalently attached to a -Gly_3Arg_7 (SEQ ID NO. 5)). The modified backbone was designated "PEIR" to denote the large nonpeptidyl carrier. Control polycation was unmodified PEI (designated "PEI", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot. The same botulinum toxin therapeutic agent was used as in example 5.

(14) Please amend the first line of page 58 of the originally filed specification as follows:
Coupling of polyethylene Imine (PEI) to TAT Fragment GGGRKKRRQRRR (SEQ ID NO. 7)

(15) Please delete paragraph [0180] of the originally filed specification and replace it with the following paragraph:

[0180] The TAT fragment GGGRKKRRQRRR (SEQ ID NO. 7) (6 mg, 0.004 mmol, Sigma Genosys, Houston, Tex.), lacking all sidechain protecting groups, was dissolved in 1 ml of 0.1 M MES buffer. To this was added EDC (3 mg, 0.016 mmol) followed by PEI 400 k molecular weight 50% solution (w:v) in water, (.about.0.02 ml, .about.2.5.times.10.sup.-5 mmol) The pH was determined to be 7.5 by test paper. Another 1 ml portion of 0.1 M MES was added and the pH was adjusted to .about.5 by addition of HCl. Another portion of EDC (5 mg, 0.026

mmol) was added and the reaction, pH.about.5 was stirred overnight. The next morning, the reaction mixture was frozen and lyophilized.

(16) Please delete paragraph [0188] of the originally filed specification and replace it with the following paragraph:

[0188] The positively charged peptidyl backbone was assembled by covalently attaching -Gly_3Arg_7 (SEQ ID NO. 5) to polylysine (MW 150,000) via the carboxyl of the terminal glycine to free amines of the lysine sidechains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is covalently attached to a -Gly_3Arg_7 (SEQ ID NO. 5)). The modified backbone was designated "KNR2". The control polycation was unmodified polylysine (designated "K2", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot.

(17) Please delete paragraph [0200] of the originally filed specification and replace it with the following paragraph:

[0200] The positively charged peptidyl backbone was assembled by covalently attaching -Arg_9 (SEQ ID NO. 8) to polylysine (MW 150,000) via the carboxyl of the terminal glycine to free amines of the lysine sidechains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is covalently attached to a -Arg_9). The modified backbone was designated "KNR". The control polycation was unmodified polylysine (designated "K", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot.